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THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

HAYSTEAD, Timothy A.

Atty. Ref.: 1579-647

Serial No.: 10/083,641

Group Art Unit:

Filed: February 27, 2002

Examiner:

For: SMOOTH MUSCLE MYOSIN PHOSPHATASE ASSOCIATED KINASE

\* \* \* \* \*

June 18, 2002

**RESPONSE TO NOTICE OF INCOMPLETE REPLY (NONPROVISIONAL)**

Hon. Commissioner of Patents  
and Trademarks  
Washington, DC 20231

Sir:

This is in response to the Notice of Incomplete Reply (NonProvisional) dated June 7, 2002, in the above matter, the period for response having been extended up to June 18, 2002 (the original due date being May 18, 2002, based on the Notice to File Missing Parts of NonProvisional Application dated March 18, 2002), by submission of the required petition and fee herewith. Kindly amend the above-identified application as follows.

**IN THE SPECIFICATION:**

Please replace the paragraph beginning at page 3, line 16, with the following rewritten paragraph:

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Fig. 2, A, B. Endogenous kinase copurifies with SMPP-1M. Autoradiography (Inset A) of purified SMPP-1M shows a phosphorylated band at 110 kDa, correlating with MYPT1. SMPP-1M was affinity purified as described (Shirazi et al, J. Biol. Chem. 269:31598-31606 (1994)) and the purified enzyme incubated with 100  $\mu$ M  $\gamma$ -[<sup>32</sup>P] ATP and 2 mM MgCl<sub>2</sub>. The reaction was terminated with sample buffer and MYPT1 resolved on SDS-PAGE gels. B. Purified M110 kinases accelerates the rate of SMPP-1M inactivation in vitro. Purified SMPP-1M was incubated for the indicated times with Mg/ATP (2mM/100  $\mu$ M) in the presence (O) or absence (●) of affinity purified M110 kinase. Note: inactivation of SMPP-1M in the absence of exogenously added M110 kinase was due to the presence of endogenous copurifying kinase activity. A. The myofibrilar extract from rabbit bladder was resolved on an AP-1Q (0.5 x 7cm) anion exchange column; the column was developed with a 0-1M NaCl gradient. SMPP-1M (O) was assayed against <sup>32</sup>P labeled myosin and SMPP-1M kinase activity (●) was assayed against the Thr<sup>697</sup> substrate peptide (KKKRQSQRRSTQGVTL) (SEQ ID NO:1).

Please replace the paragraph beginning at page 4, line 19, with the following rewritten paragraph:

Fig. 4. Identification of SMPP-1M associated kinase by mixed peptide sequencing. Mixed sequence is listed in order of the PTH amino acids recovered after each Edman cycle. Sequence data shown was derived from 200 fmol of protein. FASTF was used to search and match the mixed sequences to the NCBI/Human protein database. The scoring matrix was MD20, with expectation and score values set to <1 and 5,

respectively (Kameshita et al, Anal. Biochem. 183:139-143 (1989)). The highest scoring proteins were human ZIPK, (e) 5.1 e-14; human pDAPK3, (e) 5.1 e-14; and rat DAP-like kinase, (e) 2.1 e-7. The next highest unrelated protein score was D-glycerate dehydrogenase, (e) 0.0011 (SEQ ID NO:2 to SEQ ID NO:5).

Please replace the paragraphs beginning at page 6, line 14, with the following rewritten paragraphs:

Figure 8. Putative nucleotide sequence of the smooth muscle MYPT-kinase showing start site in bold (SEQ ID NO:6).

Figure 9. Deduced amino acid sequence of the rat aorta smooth muscle MYPT kinase (underlined shows alignment with 52 kDa ZIP kinase sequence) (SEQ ID NO:7 to SEQ ID NO:17).

Before the Figures, insert the Sequence Listing submitted herewith.

#### **REMARKS**

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

Enclosed herewith are 9 sheets of substitute drawings.

The specification has been amended to make reference to sequence identifiers and to include the Sequence Listing submitted herewith on separate sheets. Entry of the Sequence Listing does not raise the issue of new matter as the sequence information

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contained therein is presented in the application as originally filed. The computer readable copy of the Sequence Listing submitted herewith is the same as the attached paper copy of that Listing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page are captioned "Version With Markings To Show Changes Made."

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

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